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Complicating factors in the application of the “average method” for determining the contribution of gluconeogenesis

Shawn C. Burgess^{1,2}, Visvanathan Chandramouli⁴, Jeffrey D. Browning^{1,3}, William C. Schumann⁴, and Stephen F. Previs^{4,5}

¹Advanced Imaging Research Center, University of Texas Southwestern Medical Center, Dallas, Texas

²Department of Pharmacology, University of Texas Southwestern Medical Center, Dallas, Texas

³Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, Texas

⁴Department of Medicine, Case Western Reserve University, Cleveland, Ohio

⁵Department of Nutrition, Case Western Reserve University, Cleveland, Ohio

TO THE EDITOR: We are writing with regard to the study by Chacko et al. (3). Although we commend Dr. Haymond's team for addressing a difficult and important problem, the concept of using an “average” label incorporation requires additional consideration. We are concerned about whether the hydrogen/deuterium bound to carbon 1, 3, 4, 5, and 6 of glucose exchange equally during gluconeogenesis, as required for the validity of this method (3), especially since nongluconeogenic exchange reactions and/or kinetic isotope effects influence these sites differently (4,9–11). Although Chacko et al. found agreement between the hexamethylenetetramine (HMT) method and their “average method” (implying equal exchange in the various positions), the limited range of physiological conditions is understated (3). For example, Katz and coworkers (10) demonstrated that the positional labeling of newly made glucose is affected in a substrate-specific manner.

We have found that the labeling of hydrogen/deuterium bound to carbon 1 of glucose is 1.3–1.8 times that bound to carbon 6 in humans (4), and we observed time-dependent changes in the labeling ratios, e.g., the hydrogen/deuterium labeling bound to carbon 1 > carbon 5 at 14 h of fasting but approached that of carbon 5 as the fasting reached 42 h (4). Those studies relied on the HMT method (4), which in our experience (6) has better precision than that reported by Chacko et al. (3).

Nuclear magnetic resonance (NMR) spectroscopy can quantify the enrichment at all positions of glucose with an accuracy similar to the HMT method (1). Using NMR, we observed that the ²H-labeling of position 1 > position 5 > positions 3, 6_R, and 6_S in human subjects after enrichment of body water to 0.5% ²H₂O (2,5,7). We demonstrate, with ample precision, that the enrichment in these positions is significantly different in 24- and 48-h-fasted humans (Fig. 1). The reasons for differential enrichment in the various positions of

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Correspondence to: Shawn C. Burgess; Stephen F. Previs.

Address for reprint requests and other correspondence: S. C. Burgess, Advanced Imaging Research Center and Dept. of Pharmacology, Univ. of Texas S.W. Medical Center, 2201 Inwood Rd., Dallas, TX 75390-8568 (e-mail: shawn.burgess@utsouthwestern.edu) or S. F. Previs, Dept. of Nutrition-WG 48, Case Western Reserve Univ. School of Medicine, 10900 Euclid Ave., Cleveland, OH 44106-4954 (e-mail: stephen.previs@case.edu)..

glucose are well described, i.e., 1) the hydrogen on carbon 1 is exchanged during glycogenolysis and gluconeogenesis (4), 2) the hydrogen on carbon 3 is subject to a severe isotope effect during the triose isomerase reaction (9), and 3) the hydrogens on 6_R and 6_S do not include gluconeogenesis from glycerol (8). Therefore, one expects that ^2H -labeling on position 1 > position 5; and that on positions 6 and 3 are each < position 5.

The fact that the “average” method of Chacko et al. matches the 5/2 ratio (3) seems to be a fortunate concurrence, reflecting the overestimation of gluconeogenesis by position 1 and the underestimation of gluconeogenesis by positions 3, 6_R , and 6_S . We suspect that the “average” method is susceptible to systematic errors to the extent that factors controlling the enrichment of positions 1, 3, 4, 6_R , and 6_S are independent of gluconeogenesis, e.g., as gluconeogenesis approaches 100%, the accuracy of the two methods diverge (Fig. 1).

A final remark concerns the reliance of the “average method” on body water $^2\text{H}_2\text{O}$ enrichment as a surrogate for C2 enrichment. Under nonisotopic steady-state and/or “glucose-insulin” clamp conditions, the 5/2 ratio remains a valid measure of gluconeogenesis since enrichment in both positions is altered to the same degree. However, without a correction factor(s) the “average method” will underestimate gluconeogenesis because $^2\text{H}_2\text{O}$ enrichment does not change as glucose enrichment is diluted.

In summary, we concur with Chacko et al. (3), in that the HMT or NMR method requires extra consideration/instrumentation, and we commend Dr. Haymond's team for developing an alternative. However, we caution against the general application of the “average method” without broad consideration for its limitations.

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Fig. 1.

²H NMR spectra of plasma glucose (monoacetone glucose derivative) taken from a single human subject 24 and 48 h into a fast demonstrates nonequal deuterium enrichment in H1, H3, H4, H5, H6_R, and H6_S positions. The protocol and consent form were approved by the Institutional Review Board of the University of Texas Southwestern Medical Center, and all participants provided written informed consent before enrollment. Healthy lean male subjects ($n = 4$, average body mass index = 23.3) were administered enough 70% ²H₂O in 3 divided doses to enrich body water to 0.5% at 12 h into the fast. Thereafter, subjects were allowed free access to 0.5% ²H₂O as drinking water. The table shows the enrichments (means ± SE) relative to H2 after 24 and 48 h of fasting. In all cases, H1 was more enriched than H5, while H3, H6_R, and H6_S were always less enriched than H5; only H4 was similarly enriched as H5. Fractional gluconeogenesis was determined either by H5/H2 or by taking the average (H1, H3, H5, H6_R, H6_S)/H2, analogous to Chacko et al. (3). The 2 methods gave similar results after a 24-h but not a 48-h fast, indicating that under some conditions, overenrichment of H1 in combination with the underenrichment of H3, H6_R, and H6_S, when taken as an average, can provide similar values of gluconeogenesis as the H5/H2 method. However, when gluconeogenesis approaches 100%, the “average” method fails to match the H5/H2. Significant differences between H5 enrichment and other positions or between the 2 methods were tested using a paired Student's *t*-test. NA, not applicable.